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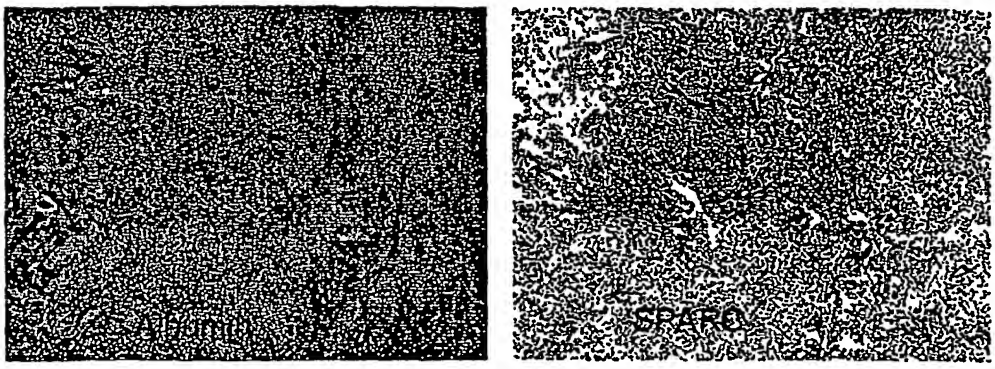
(54) Title: TREATMENT METHODS UTILIZING ALBUMIN-BINDING PROTEINS AS TARGETS



Colocalization of albumin and SPARC in serial sections of an MX-1 xenograft tumor.

(57) Abstract: The invention provides a method for determining the response of a mammalian tumor to a chemotherapeutic agent comprising detecting and quantifying the SPARC protein in a sample isolated from the human. The invention also provides a method for delivering a chemotherapeutic agent to a tumor in a human that expresses SPARC comprising administering a pharmaceutical composition comprising the chemotherapeutic agent coupled to a compound that binds SPARC protein. The invention further provides a composition comprising a chemotherapeutic agent coupled to a compound capable of binding SPARC protein and a pharmaceutically acceptable carrier.

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TREATMENT METHODS UTILIZING ALBUMIN-BINDING PROTEINS AS TARGETS

FIELD OF THE INVENTION

[0001] This invention relates to methods for treating cancer; as well as other diseases involving abnormal proliferative, hyperplastic, remodeling, inflammatory activity in tissues and organs. The invention further relates to methods for targeting, imaging, and determining the response of mammalian tumors to a chemotherapeutic agent using suitable ligands recognizing albumin-binding proteins.

BACKGROUND OF THE INVENTION

[0002] Albumin nanoparticle formulations have been shown to reduce toxicity of poorly soluble therapeutic agents. For example, U.S. Patent 6,537,579 discloses an albumin-nanoparticle paclitaxel formulation which is free of toxic emulsifiers.

[0003] The anticancer agent paclitaxel, marketed under the trademark Taxol® by Bristol Myers Squibb, is currently approved for the treatment of several cancers including ovarian, lung, and breast cancer. The major limitation to the use of paclitaxel is its poor solubility. Consequently, the Taxol® formulation contains Cremophor® EL as the solubilizing vehicle, but the presence of Cremophor® in this formulation has been linked to severe hypersensitivity reactions in animals (Lorenz et al., Agents Actions 7, 63-67, 1987), and humans (Weiss et al., J. Clin. Oncol. 8, 1263-1268, 1990). Accordingly, patients receiving Taxol® require premedication with corticosteroids (dexamethasone) and antihistamines to reduce the hypersensitivity and anaphylaxis that occurs due to the presence of Cremophor®.

[0004] In contrast, Abraxane® also known as ABI-007 is a Cremophor®-free, albumin-nanoparticle formulation of paclitaxel, marketed by Abraxis Oncology. The use of an albumin nanoparticle as a vehicle results in the formation of a colloid when reconstituted with saline. Based on clinical studies, it has been shown that the use of Abraxane® is characterized by reduced hypersensitivity reactions as compared with Taxol®. Accordingly, premedication is not required for patients receiving Abraxane®.

[0005] Another advantage of the albumin-nanoparticle formulation is that by excluding toxic emulsifiers it is possible to administer higher doses of paclitaxel at more frequent intervals than is currently possible with Taxol®. The potential exists that enhanced efficacy could be seen in solid tumors as a consequence of (i) higher tolerable doses (300 mg/m^2), (ii) longer half-life, (iii) prolonged local tumor availability and/or (iv) sustained *in vivo* release. Abraxane® reduces hypersensitivity reactions while maintaining or improving the chemotherapeutic effect of the drug.

[0006] It is known that colloidal nanoparticles or particles <200 nm in size tend to concentrate at the tumor site due to leaky vasculatures. This effect has been described for several lipsomal formulations (Papahadjopoulos, et al., Proc. Natl. Acad. Sci. U.S.A. 88, 11460, 1991); (Gabizon, A., Cancer Res., 52, 891, 1992); (Dvorak, et al., Am. J. Pathol. 133, 95, 1988); (Dunn, et al., Pharm, Res., 11, 1016-1022, 1994); and (Gref, et al, Science 263, 1600-1603, 1994). It is possible that localized nanoparticles of paclitaxel at the tumor site may result in slow release of the drug at the tumor site resulting in greater efficacy when compared to administration of the drug in its solubilized (Cremophor®-containing) form.

[0007] Such nanoparticle formulations comprise at least about 50% of the active agent in nanoparticle form. Further, such nanoparticle formulations comprise at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the active agent in nanoparticle form. Moreover, such nanoparticle formulations comprise at least about 95% or at least about 98% of the active agent in nanoparticle form.

[0008] Secreted Protein, Acidic, Rich in Cysteines (SPARC), also known as osteonectin, is a 281 amino acid glycoprotein. SPARC has affinity for a wide variety of ligands including cations (e.g., Ca^{2+}, Cu^{2+}, Fe^{2+}), growth factors (e.g., platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF)), extracellular matrix (ECM) proteins (e.g., collagen I-V and collagen IX, vitronectin, and thrombospondin-1), endothelial cells, platelets, albumin, and hydroxyapaptite. SPARC expression is developmentally regulated, and is predominantly expressed in tissues undergoing remodeling during normal development or in response to injury (see, e.g., Lane et al., *FASEB J.*, *8*, 163-173 (1994)). High levels of SPARC protein are expressed in developing bones and teeth.

[0009] SPARC is a matricellular protein upregulated in several aggressive cancers, but is absent in normal tissues (Porter et al., *J. Histochem. Cytochem.*, *43*, 791(1995)). Indeed, SPARC expression is induced among a variety of tumors (e.g., bladder, liver, ovary, kidney, gut, and breast). In bladder cancer, for example, SPARC expression has been associated with advanced carcinoma. Invasive bladder tumors of stage T2 or greater have been shown to express higher levels of SPARC than bladder tumors of stage T1 (or less superficial tumors), and have poorer prognosis (see, e.g., Yamanaka et al., *J. Urology*, *166*, 2495-2499 (2001)). In meningiomas, SPARC expression has been associated with invasive tumors only (see, e.g., Rempel et al., *Clincal Cancer Res.*, *5*, 237-241 (1999)). SPARC expression also has been detected in 74.5 % of *in situ* invasive breast carcinoma lesions (see, e.g., Bellahcene, et al., *Am. J. Pathol.*, *146*, 95-100 (1995)), and 54.2% of infiltrating ductal carcinoma of the breast (see, e.g., Kim et al., *J. Korean Med. Sci.*, *13*, 652-657 (1998)).

SPARC expression also has been associated with frequent microcalcification in breast cancer (see, e.g., Bellahcene et al., *supra*), suggesting that SPARC expression may be responsible for the affinity of breast metastases for the bone. SPARC is also known to bind albumin (see, e.g., Schnitzer, *J. Biol. Chem.*, *269*, 6072 (1994)).

[0010] Antibody therapy is an effective method for controlling disease wherein a specific protein marker can be identified. Examples include Avastin – an anti-VEGF antibody, Rituxan – an anti-CD20 antibody, and Remicade – an anti-TNF antibody. As such, an antibody against SPARC would represent an important therapeutic agent for treating human and other mammalian tumors, as well as other proliferative, hyperplastic, remodeling, and inflammatory disorders that express the SPARC protein. In addition, an antibody against SPARC conjugated with an imaging or contrast agent would be a means of detecting and diagnosing such disorders.

[0011] There remains a need for a method of treating human and other mammalian tumors, as well as other proliferative, hyperplastic, remodeling, and inflammatory disorders. Moreover, there remains a need for determining the response of a human or other mammalian tumor in order to evaluate the effectiveness of the chemotherapeutic agent. In addition, suitable means are needed in order to detect and diagnose such disorders. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

[0012] The invention provides a method for determining the response of a mammalian tumor to a chemotherapeutic agent, wherein the method comprises the steps of (a) isolating a biological sample from the mammal, (b) detecting the expression of SPARC protein in the biological sample, and (c) quantifying the amount of SPARC protein in the biological sample. The invention further provides a method for utilizing the albumin-binding protein as a therapeutic, for utilizing SPARC and antibodies against SPARC or SPARC binding-proteins as therapeutics, for delivering a chemotherapeutic agent to a disease site in a mammal, in which the method comprises administering to the mammal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises the chemotherapeutic agent coupled to a compound capable of binding an albumin-binding protein and a pharmaceutically acceptable carrier. In addition, the invention provides a composition comprising a chemotherapeutic agent coupled to a compound capable of binding a SPARC protein and a pharmaceutically acceptable carrier. Moreover, the invention provides a delivery agent comprising a SPARC recognition group and a therapeutic agent, wherein the therapeutic agent is coupled to the SPARC recognition

group. Furthermore, the invention provides a method for delivering a chemotherapeutic agent to a tumor in a mammal, wherein the method comprises administering to a mammal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises a chemotherapeutic agent coupled to a SPARC protein capable of binding albumin and a pharmaceutically acceptable carrier. The invention compositions may be small molecule, large molecule or proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 illustrates albumin and SPARC staining in MX-1 tumor xenografts.

[0014] Figure 2 illustrates transcytosis of paclitaxel across endothelial cell monolayers.

DETAILED DESCRIPTION OF THE INVENTION

[0015] It has now been discovered that an additional mechanism of localization exists for compositions comprising albumin-binding proteins. Albumin-binding proteins, such as SPARC, cubilin, and TGFβ, can be used to target a therapeutic agent to a disease site characterized by an overexpression of an albumin-binding protein.

[0016] The invention provides for the use of a group, coupled to an agent, wherein the group is capable of binding an albumin-binding protein, such as SPARC, cubilin, or TGFβ, and the agent is a therapeutic, imaging, or delivery agent for diseases wherein the albumin-binding protein plays a dominant role and is overexpressed relative to normal tissues. Preferably, the albumin-binding protein is selected from SPARC, cubilin, or TGFβ. Most preferred, the albumin-binding protein is SPARC and the group which binds the albumin-binding protein is a SPARC recognition group. Suitable SPARC recognition groups include, but are not limited to ligands, small molecules, antibodies.

[0017] The invention also provides a method for determining the response of a human or other mammalian tumor to a chemotherapeutic agent. The method comprises (a) isolating a biological sample from the human, (b) detecting the expression of SPARC protein in the biological sample, and (c) quantifying the amount of SPARC protein in the biological sample. Once the amount of SPARC expressed by the tumor is determined, the effectiveness of the chemotherapeutic agent can be ascertained by, for example, correlating of the expression of SPARC to the dosage of therapeutic agent administered. The invention also provides for the use of antibody raised against SPARC as a therapeutic agent, or an imaging agent for diseases where SPARC plays a dominant role and is overexpressed relative to normal tissues.

[0018] Hereinafter, for simplicity, all proteins, including SPARC, that bind albumin are referred to as SPARC. The SPARC protein is responsible for the accumulation of albumin

in certain human tumors. As albumin is the major carrier of chemotherapeutic drugs, the expression level of SPARC is indicative of the amount of chemotherapeutic drug that penetrates and is retained by the tumor. Therefore, the expression level of SPARC is predictive of the responsiveness of the tumor to chemotherapy.

[0019] Any suitable biological sample can be isolated from the mammal of interest in the context of the inventive method. Preferably, the biological sample is isolated from the tumor, such as by a tumor biopsy. Alternatively, the biological sample can be isolated from a bodily fluid of the mammal, including, for example, cerebrospinal fluid, blood, plasma, serum, or urine. Techniques and methods for the isolation of biological samples are known to those in the art.

[0020] Any suitable pharmaceutically active agent can be used in the inventive method (e.g., a chemotherapeutic agent coupled to a SPARC recognition group), so long as the transport or binding of the active agent requires albumin. Suitable active agents include, but are not limited to, tyrosine kinase inhibitors (genistein), biologically active agents (TNF or tTF), radionuclides (e.g., ^{131}I, ^{90}Y, ^{111}In, ^{211}At, ^{32}P, and other known therapeutic radionuclides), adriamycin, ansamycin antibiotics, asparaginase, bleomycin, busulphan, cisplatin, carboplatin, carmustine, capecitabine, chlorambucil, cytarabine, cyclophosphamide, camptothecin, dacarbazine, dactinomycin, daunorubicin, dexrazoxane, docetaxel, doxorubicin, etoposide, epothilones, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine, mechlorethamine, mercaptopurine, meplhalan, methotrexate, rapamycin (sirolimus) and derivatives, mitomycin, mitotane, mitoxantrone, nitrosurea, paclitaxel, pamidronate, pentostatin, plicamycin, procarbazine, rituximab, streptozocin, teniposide, thioguanine, thiotepa, taxanes, vinblastine, vincristine, vinorelbine, taxanes, combretastatins, discodermolides, transplatinum, vascular targeting agents, anti-vascular endothelial growth factor compounds ("anti-VEGFs"), anti-epidermal growth factor receptor compounds ("anti-EGFRs"), 5-fluorouracil, and derivatives thereof.

[0021] In addition, the pharmaceutically active agent can be toxins such as ricin A, radionuclides, the Fc fragment of the antibody itself, single chain antibodies, Fab fragments, diabodies, and the like. The pharmaceutically active agents selected themselves can recognize and bind to SPARC or are suitably attached to a SPARC recognition group that recognizes SPARC, including, for example, a protein or non-protein, an antibody, Fc fragment of the antibody itself, single chain antibodies, Fab fragments, diabodies, peptides, or other non-protein small molecules.

[0022] One or more doses of one or more chemotherapeutic agents can be administered according to the inventive method. The type and number of chemotherapeutic agents used

in the inventive method will depend on the standard chemotherapeutic regimen for a particular tumor type. In other words, while a particular cancer may be treated routinely with a single chemotherapeutic agent, another may be treated routinely with a combination of chemotherapeutic agents. Methods for coupling or conjugation of suitable therapeutics, chemotherapeutics, radionuclides, etc. to antibodies or fragments thereof are well described in the art.

[0023] Diseases for which the present invention is useful include abnormal conditions of proliferation, tissue remodeling, hyperplasia, exaggerated wound healing in any bodily tissue including soft tissue, connective tissue, bone, solid organs, blood vessel and the like. Examples of diseases treatable or diagnosed by invention compositions include cancer, diabetic or other retinopathy, inflammation, arthritis, restenosis in blood vessels or artificial blood vessel grafts or intravascular devices and the like.

[0024] The types of tumor to be detected and treated in accordance with the invention are generally those found in humans and other mammals. The tumors can be the result of inoculation as well, such as in laboratory animals. Many types and forms of tumors are encountered in human and other animal conditions, and there is no intention to limit the application of the methods of the present to any particular tumor type or variety. Tumors, as is known, include an abnormal mass of tissue that results from uncontrolled and progressive cell division, and is also typically known as a "neoplasm." The inventive methods are useful for tumor cells and associated stromal cells, solid tumors and tumors associated with soft tissue, such as, soft tissue sarcoma, for example, in a human. The tumor or cancer can be located in the oral cavity and pharynx, the digestive system, the respiratory system, bones and joints (e.g., bony metastases), soft tissue, the skin (e.g., melanoma), breast, the genital system, the urinary system, the eye and orbit, the brain and central nervous system (e.g., glioma), or the endocrine system (e.g., thyroid) and is not necessarily limited to the primary tumor or cancer. Tissues associated with the oral cavity include, but are not limited to, the tongue and tissues of the mouth. Cancer can arise in tissues of the digestive system including, for example, the esophagus, stomach, small intestine, colon, rectum, anus, liver, gall bladder, and pancreas. Cancers of the respiratory system can affect the larynx, lung, and bronchus and include, for example, non-small cell lung carcinoma. Tumors can arise in the uterine cervix, uterine corpus, ovary vulva, vagina, prostate, testis, and penis, which make up the male and female genital systems, and the urinary bladder, kidney, renal pelvis, and ureter, which comprise the urinary system. The tumor or cancer can be located in the head and/or neck (e.g., laryngeal cancer and parathyroid cancer). The tumor or cancer also can be located in the hematopoietic system or lymphoid system, and include, for example, lymphoma (e.g., Hodgkin's disease and

Non-Hodgkin's lymphoma), multiple myeloma, or leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, and the like). Preferably, the tumor is located in the bladder, liver, ovary, kidney, gut, brain, or breast.

[0025] The SPARC protein has affinity for a wide variety of ligands. Thus, the inventive method for delivering a therapeutic agent to a site of disease is predicated on the discovery that compounds or ligands, including albumin, which have affinity for SPARC can be used to deliver therapeutic drugs to the site of disease, with little or no delivery to normal tissues.

[0026] The invention also provides for a means of transporting the therapeutic composition across the endothelial barrier from the blood vessel into the tumor interstitium. The main hurdle in antibody therapy and chemotherapy is the translocation across the endothelial barrier into tumor interstitium. Albumin utilizes the albumin receptor transport mechanism to cross the endothelial barrier. This transport mechanism could be the same as those reported by the literature (gp60 and albondin) or by other undiscovered mechanisms. It has been previously reported that the therapeutic agent piggy backed onto albumin exhibited enhanced tumoral uptake (Desai, N. et al. Increased endothelial transcytosis of nanoparticle albumin-bound paclitaxel (ABI-007) by endothelial gp60 receptors: a pathway inhibited by Taxol®, 27th Annual San Antonio Breast Cancer Symposium (SABCS) (2004), abstract #1071). Further, enhanced translocation across the endothelial barrier can be achieved using the physiological albumin transport mechanism (Schnitzer, J.E.; Oh, P. J. Biol. Chem. 269, 6072-6082 (1994).

[0027] For small molecules, modifications can be made so that the drug affinity for albumin is increased. For formulations of small molecules, a solvent which prevents the binding of the drug to albumin may be removed. Alternatively, the small molecule may be linked to albumin, antibody against albumin, fragments thereof or ligands for an albumin-receptor such as described below.

[0028] For biologic molecules such as proteins, antibodies and fragments thereof, it is possible to engineer the biologics with an albumin binding peptide such that the biologics will exhibit an affinity for albumin. The peptide can either be an albumin binding sequence, an antibody or antibody fragment against albumin, antibody or antibody fragment against albumin carriers (such as gp60/albondin/scavenger receptor/or TGF-beta receptor), or antibody to any of the proteins found in the caveolae, the transporter of albumin. The invention also contemplates an antibody or suitable fragment thereof prepared as a chimera with one valence for SPARC and another valence for an effector of transendothelial

transport such as gp60/albondin/ scavenger receptor/or TGF-beta receptor, or against any of the proteins found in the caveolae of the endothelial cell.

[0029] The invention also provides a method for the destruction of SPARC expression tissues such as tumor and restenotic tissues via the complement fixation and/or recruitment of cell mediated immune response by SPARC antibody. In this case, like that of Rituxan, an anti-CD20 antibody, the effector moiety is the Fc fragment which can mediate either complement activation with direct destruction of SPARC expression cells or recruitment of immune cells to the SPARC expression tissue with resulting tissue destruction via a cell mediated immune response.

[0030] The invention also provides a method for inhibition of SPARC activity using a neutralizing antibody against SPARC. The neutralizing antibody has the ability to block the interaction of SPARC with its effectors *in vivo*. For example, the neutralizing antibody may block interaction of SPARC with a cell surface component or the binding of SPARC to its natural ligands such as albumin, growth factors, and Ca^{2+}.

[0031] The invention also provides a method for determining the response of a human or other mammalian tumor to anti-SPARC therapy. The method comprises (a) isolating a biological sample from the human, (b) detecting the expression of SPARC protein in the biological sample, and (c) quantifying the amount of SPARC protein in the biological sample. As anti-SPARC therapy relies on the binding of SPARC antibody to SPARC in disease tissue, the presence of SPARC in disease tissue is necessary for activity.

[0032] The invention further provides a method of using one or more diagnostic agents conjugated to the SPARC recognition groups, such as the antibodies or fragments thereof, as described above. The diagnostic agents include radioisotopes or radionuclides, MRI contrast agents, X-ray contrast agents, ultrasound contrast agents and PET contrast agents. Methods utilized for conjugation are known in the art.

[0033] The expression of SPARC protein in a sample can be detected and quantified by any suitable method known in the art. Suitable methods of protein detection and quantification include Western blot, enzyme-linked immunosorbent assay (ELISA), silver staining, the BCA assay (Smith et al., *Anal. Biochem.*, *150*,76-85 (1985)), the Lowry protein assay (described in, e.g., Lowry et al., *J. Biol. Chem.*, *193*, 265-275 (1951)), which is a colorimetric assay based on protein-copper complexes, and the Bradford protein assay (described in, e.g., Bradford et al., *Anal. Biochem.*, *72*, 248 (1976)), which depends upon the change in absorbance in Coomassie Blue G-250 upon protein binding. Tumor biopsy can be analyzed by any of the preceeding methods or it can be analyzed by immunohistochemistry using anti-SPARC antibody (either monoclonal or polyclonal) in

conjunction with appropriate visualization system (i.e., HRP substrate and HRP-conjugated secondary antibody).

[0034] Any suitable antibodies against SPARC can be used in the inventive method, so long as the antibody exhibits specific binding to SPARC. The antibody can either be monoclonal or polyclonal; and can be produced either through immunization of an animal or produced through recombinant DNA technology such as phage display and *in vitro* mutagenesis or synthesis of the variable regions of the antibody heavy and light chain genes. Polyclonal antibodies include, but are not limited to human antibodies and humanized antibodies derived from animals such as avian (e.g, chicken), rodent (e.g., rat, mouse, hamster, guinea pig), cow, goat, sheep, rabbit and the like. Monoclonal antibodies include antibodies derived from a single clone of antibody producing cells including, but not limited to, human cells, and antibodies derived from the cells of other animal types, for example, chicken, rabbit, rat, mouse, hamster, guinea pig, cow, goat, sheep, and the like. Synthetic antibodies include antibodies produced using recombinant DNA technology via genetic engineering of the variable regions of the heavy and light chain genes. Synthetic antibodies also include chemically synthesized antibody fragments with SPARC binding activity or antibodies derived from phage display or similar technology.

[0035] For human use, in order to avoid immunogenicity and immune response, it is preferable to use humanized anti-SPARC antibody or suitable fragments such as Fab', Fab, or Fab2. Humanized antibody or fragments thereof maybe produced, for example, using one of the following established methods: 1) humanized antibody may be constructed using human IgG backbone replacing the variable CDR region with that of antibody against SPARC, where the heavy and light chain are independently expressed under separate promoters or coexpressed under one promoter with IRES sequence; 2) humanized monoclonal antibody may be raised against SPARC using a mouse engineered to have a human immune system; 3) humanized antibody against SPARC may be raised using phagemid (M13, lambda coliphage, or any phage system capable of surface presentation). To construct the full length antibody, the variable region may be transferred onto the CDR of both Heavy chain and Light chain. The coexpression of the Heavy chain and Light Chain in mammalian cells such as CHO, 293, or human myeloid cells results in full length antibody. Similarly, Fab', Fab, or Fab2 fragments and single chain antibodies can be prepared using well established methods.

[0036] Antibody against SPARC is also not limited to whole antibody or fragment of the antibody retaining the binding site for SPARC (e.g., Fab and Fab2). The antibody is also not limited to any one class of antibody, e.g., IgM, IgA, IgG, IgE, IgD, and IgY. The antibody is also not limited to divalent antibody, monovalent, or chimera with one valence

for SPARC and another for an effector such tTF or ricin A. The humanized antibody is not limited to IgG. The same technologies can be used to generate all other classes of antibodies such as IgE, IgA, IgD, IgM, each having different antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) activities appropriate to particular disease target. Functional fragments of the antibody can be generated by limited proteolysis. These fragments can be monovalent such as Fab' or divalent, such as Fab2. Fragments can also be synthesized as single chain scfv or diabodies in *E. coli.*

[0037] The invention further provides a composition comprising a pharmaceutically active agent directly able to exert its pharmacological effect or a pharmaceutically active agent coupled to a compound capable of binding SPARC, or other or other albumin binding moiety, and a pharmaceutically acceptable carrier. The delivery agent, which may be a pharmaceutical composition, comprise the pharmaceutically active agent coupled to a SPARC recognition group is administered to a mammal, such as a human, in an amount such that a therapeutically effective amount of the pharmaceutically active agent is delivered to the mammal.

[0038] The invention also provides a method for delivering a chemotherapeutic agent to a tumor in a mammal. The method comprises administering to a human or other mammal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises the chemotherapeutic agent coupled to a compound or ligand capable of binding a SPARC protein and a pharmaceutically acceptable carrier. Descriptions of the chemotherapeutic agent, tumor, mammal, and components thereof, set forth herein in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a chemotherapeutic agent to a tumor.

[0039] Preferably, the pharmaceutical composition does not comprise more than 50% of the therapeutic agent in nanoparticle form. More preferably, the pharmaceutical composition does not comprise more than 10% of the therapeutic agent in nanoparticle form. Even more preferably, the pharmaceutical composition does not comprise more than 5%, or more than 4% or more than 3% of the therapeutic agent in nanoparticle form. In a more preferred embodiment, the pharmaceutical composition does not comprise more than 2% or, more than 1% of the therapeutic agent in nanoparticle form. Most preferably, the pharmaceutical composition does not comprise any of the therapeutic agent in nanoparticle form.

[0040] The invention also provides a method for delivering a chemotherapeutic agent to a tumor in a human or other mammal. The method comprises administering to a human or other mammal a therapeutically effective amount of a delivery agent, such as a

pharmaceutical composition, wherein the delivery agent (e.g., pharmaceutical composition) comprises the chemotherapeutic agent coupled to the SPARC recognition group. For example, the chemotherapeutic agent can be coupled to a SPARC recognition group such as an antibody recognizing SPARC protein, or the SPARC antibody alone. Pharmaceutical compositions preferably include the chemotherapeutic agent coupled to the SPARC recognition group and a pharmaceutically acceptable carrier. Descriptions of the chemotherapeutic agent, tumor, mammal, and components thereof, set forth herein in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a chemotherapeutic agent to a tumor.

[0041] In other embodiments, the invention provides a method for delivering a pharmaceutically active agent by way of a SPARC recognition group to a site of disease that is characterized by overexpression of SPARC or another albumin-binding protein or marker in a human, or other animal that expresses such protein or marker. Such diseases include abnormal conditions of proliferation, tissue remodeling, hyperplasia, and exaggerated wound healing in bodily tissue (e.g., soft tissue, connective tissue, bone, solid organs, blood vessel and the like). Examples of diseases that are treatable or may be diagnosed by administering a pharmaceutical composition comprising a therapeutic agent coupled to a compound or ligand capable of binding a SPARC protein, or another albumin-binding protein, include cancer, diabetic or other retinopathy, inflammation, arthritis, restenosis in blood vessels, artificial blood vessel grafts, or intravascular devices, and the like. Descriptions of the pharmaceutically active agent, tumor, mammal, and components thereof, set forth herein in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a pharmaceutically active agent.

[0042] In yet other embodiments, the invention provides a method for delivering a pharmaceutically active agent (for example, SPARC antibody alone or chemotherapeutic agent conjugated to SPARC recognition group such as SPARC antibody, radiolabelled SPARC antibody and the like) to a site of disease that is characterized by overexpression of SPARC in a human, or other animal that expresses such protein or marker. Such diseases include abnormal conditions of proliferation, tissue remodeling, hyperplasia, and exaggerated wound healing in bodily tissue (e.g., soft tissue, connective tissue, bone, solid organs, blood vessel and the like). Examples of diseases that are treatable or diagnosed by administering a pharmaceutical composition comprising anti-SPARC therapy, include cancer, diabetic or other retinopathy, inflammation, arthritis, restenosis in blood vessels, artificial blood vessel grafts, or intravascular devices, and the like. Descriptions of the pharmaceutically active agent, tumor, mammal, and components thereof, set forth here in

connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a pharmaceutically active agent.

[0043] In other embodiments, the inventive method comprises administering to a mammal a therapeutically effective amount of a pharmaceutical composition comprising a chemotherapeutic agent coupled to a compound or ligand capable of binding SPARC protein. The chemotherapeutic agent can be coupled to the compound or ligand capable of binding SPARC protein using any suitable method. Preferably, the chemotherapeutic agent is chemically coupled to the compound via covalent bonds including, for example, disulfide bonds.

[0044] The invention also provides a method comprising administering to a mammal a therapeutically effective amount of a pharmaceutical composition comprising a chemotherapeutic agent or radioactive element coupled to a SPARC recognition group. The chemotherapeutic or radioactive agent can be coupled to the antibody recognizing SPARC using any suitable method. Preferably, the chemotherapeutic agent can be chemically coupled to the compound via covalent bonds including, for example, disulfide bonds.

[0045] Preferably, the compound or ligand useful in the inventive method is capable of binding the SPARC protein. In a preferred embodiment of the invention, the compound is a ligand that binds a SPARC protein. Examples of suitable ligands include calcium cation (Ca^{2+}), copper cation (Cu^{2+}), iron cation (Fe^{2+}), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), collagen (e.g., collagen I, collagen II, collagen III, collagen IV, collagen V, and collagen IX), vitronectin, thrombospondin-1, endothelial cells, platelets, albumin, and hydroxyapatitecations. In another preferred embodiment of the invention, the compound is a small molecule. The term "small molecule" refers to any molecule having a molecular weight less than about 600. Examples of suitable small molecules include a protein, a nucleic acid, a carbohydrate, a lipid, a coenzyme (e.g., a vitamin), an antigen, a hormone, and a neurotransmitter. Preferably, the small molecule is a chemical (e.g., an organic or inorganic chemical) a peptide, or peptide mimetic, a protein, or a carbohydrate. In yet another preferred embodiment of the invention, the compound is an antibody that is directed against a SPARC protein. Any suitable antibody, or fragment thereof, that binds to a SPARC protein can be used in the inventive method.

[0046] SPARC expression in tumor tissues has been demonstrated in almost all cancer types. There has been evidence that elaboration of SPARC on tumor tissues can be either derived from tumoral expression of SPARC or by stromal cells. The SPARC phenotype of the tumor can be converted from SPARC negative to SPARC positive by administration of exogenous SPARC. Accordingly, the SPARC positive tumor would then become sensitive

to chemotherapeutic agents. Alternatively, SPARC could be radiolabeled or conjugated with various toxins to confer on it the ability to kill the tumor directly or indirectly.

[0047] SPARC can be synthesized and purified using known technologies. Cells expressing exogenous SPARC can be generated by placing the SPARC structural gene/cDNA under the control of strong promoter/translation start and the vector transfected into mammalian cells to drive the expression of SPARC in these cells. Alternatively, SPARC can be expressed using bacculovirus or other viruses such as adenovirus. SPARC expressed by these cells can be purified by traditional purifications methods such as ionic exchange, size exclusion, or C18 chromatography. The purified SPARC can be formulated in saline with preservatives and administered intravenously, by aerosol, by subcutaneous injection, or other methods.

[0048] The invention also provides a method for delivering a chemotherapeutic agent to a tumor in a mammal. The method comprises administering to a mammal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises the chemotherapeutic agent coupled to a SPARC protein capable of binding albumin and a pharmaceutically acceptable carrier. Descriptions of the chemotherapeutic agent, tumor, mammal, and components thereof, set forth herein in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a chemotherapeutic agent to a tumor.

[0049] For use *in vivo*, the chemotherapeutic agent coupled to a compound or ligand capable of binding the SPARC protein desirably is formulated into a pharmaceutical composition comprising a physiologically acceptable carrier. Any suitable physiologically acceptable carrier can be used within the context of the invention, and such carriers are well known in the art.

[0050] For use *in vivo*, the anti-SPARC therapy agent desirably is formulated into a pharmaceutical composition comprising a physiologically acceptable carrier. Any suitable physiologically acceptable carrier can be used within the context of the invention, and such carriers are well known in the art.

[0051] The carrier typically will be liquid, but also can be solid, or a combination of liquid and solid components. The carrier desirably is a physiologically acceptable (e.g., a pharmaceutically or pharmacologically acceptable) carrier (e.g., excipient or diluent). Physiologically acceptable carriers are well known and are readily available. The choice of carrier will be determined, at least in part, by the location of the target tissue and/or cells, and the particular method used to administer the composition.

[0052] Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions

upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified. The pharmaceutical formulations suitable for injectable use include sterile aqueous solutions or dispersions; formulations containing known protein stabilizers and lyoprotectants, formulations including sesame oil, peanut oil or aqueous propylene glycol, and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the formulation must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxycellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0053] The chemotherapeutic agent (e.g., anti-SPARC therapy) coupled to a compound or ligand which binds a SPARC protein can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such as organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0054] The composition can further comprise any other suitable components, especially for enhancing the stability of the composition and/or its end-use. Accordingly, there is a wide variety of suitable formulations of the composition of the invention. The following formulations and methods are merely exemplary and are in no way limiting.

[0055] Formulations suitable for administration via inhalation include aerosol formulations. The aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as non-pressurized preparations, for delivery from a nebulizer or an atomizer.

[0056] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can

be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. In a preferred embodiment of the invention, the chemotherapeutic agent coupled to a compound which binds a SPARC protein (e.g., anti-SPARC therapy) is formulated for injection (e.g., parenteral administration). In this regard, the formulation desirably is suitable for intratumoral administration, but also can be formulated for intravenous injection, intraperitoneal injection, subcutaneous injection, and the like.

[0057] Formulations suitable for anal administration can be prepared as suppositories by mixing the active ingredient with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0058] In addition, the composition can comprise additional therapeutic or biologically-active agents. For example, therapeutic factors useful in the treatment of a particular indication can be present. Factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with *in vivo* administration of the pharmaceutical composition and physiological distress.

[0059] The following examples further illustrate the invention but should not be construed as in any way limiting its scope.

EXAMPLE 1

[0060] This example illustrates the co-localization of SPARC with albumin in an MX-1 tumor xenograft.

[0061] Paclitaxel albumin nanoparticles (Abraxane, ABX or ABI-007) have been shown to have an improved response rate over Taxol (TAX) in a Phase 3 metastatic breast cancer trial (33% vs. 19%, $p<0.0001$) (see, e.g., O'Shaughnessy, SABCS). Albumin-mediated transendothelial transport of paclitaxel (P) and increased intratumoral accumulation of paclitaxel for ABX versus TAX was demonstrated recently (see, e.g., Desai, SABCS 2003). Albumin binds to SPARC (see, e.g., Schnitzer, *J. Biol. Chem.*, *269*, 6072-82 (1994)).

[0062] The MX-1 tumor cell line is derived from a human breast cancer. Serial cryosections of human MX-1 tumor xenograft, human primary breast tumor tissues (n=141), and normal human breast tissue (n=115) were immunostained and scored (0-4) for albumin, SPARC (using anti-SPARC antibody), and caveolin-1 staining. Cultured MX-1

cells also were immunostained for SPARC. Paclitaxel albumin nanoparticles (Abraxane, ABX or ABI-007) and Taxol (TAX) were prepared with radioactive paclitaxel (P) (20 mg/kg IV), and were used to determine the biodistribution of paclitaxel in normal tissues of athymic mice.

[0063] Albumin staining in the MX-1 tumor was focal and co-localized with SPARC (Figure 1). Caveolin-1 staining confirmed that blood vessel density in albumin-containing areas was no different from albumin-free areas. SPARC expression by MX-1 cultured cells was confirmed by positive staining with anti-SPARC antibody. Paclitaxel accumulation in normal tissues (SPARC negative) was significantly lower for ABX as compared to TAX ($p \leq 0.004$) for 7/10 tissues. 46% of the human primary breast tumors exhibited strong SPARC staining (score ≥ 2), as compared to 1% for normal tissues ($p < 0.0001$). In a subset of 50 tumor tissues, SPARC expression did not correlate with staging, ER status, or PgR status; however, there was trend for high SPARC expression among p53-negative tumors.

[0064] The co-localization of albumin and SPARC suggests that SPARC, by its albumin binding activity, may behave as an intratumoral target for albumin binding in breast tumors. As transport of paclitaxel in ABX is dependent on albumin (see, e.g., Desai SABCS, 2003), this may explain the improved tumor accumulation of ABX as compared to TAX. ABX accumulation in normal tissues was lower than for TAX, consistent with lack of SPARC expression in normal tissues. Screening of patients for SPARC allows for the identification of patients more responsive to ABX. The presence of SPARC in these tumors allows for targeting and therapy using anti-SPARC antibody.

EXAMPLE 2

[0065] This example illustrates the expression of SPARC in MX-1 tumor cells.

[0066] MX-1 cells were cultured on a coverslip and stained with an antibody directed against human SPARC using methods known in the art. Antibody staining was observed, which demonstrates that MX-1 is expressing SPARC. These results suggest that SPARC expression detected in MX-1 tumor cells is a result of SPARC secretion by MX-1 tumor cells. Staining was more intense for MX-1 tumor cells than that of normal primary cells such as HUVEC (human umbiblical vein endothelial cells), HLMVEC (Human lung microvessel endothelial cells) , and HMEC (Human mammary epithelial cells). Though the majority of the SPARC staining was internal SPARC, significant level of surface SPARC was detected as demonstrated by confocal miscroscopy and staining of unpermeabilized cells.

EXAMPLE 3

[0067] This example illustrates the overexpression of SPARC protein in human breast carcinoma cells.

[0068] SPARC expression in human breast carcinoma cells was determined using a tumor array from Cybrdi, Inc. (Gaithersburg, MD). The results of this analysis are set forth in Table 1. Intensity of staining was scored from "Negative" to 4+, with the higher number corresponding to greater intensity of overexpression. 49% of breast carcinoma stained positive (2+ and above) for SPARC, as compared to 1% of normal tissue ($p<0.0001$).

	SPARC Staining (%)					
	Negative	-/+	1+	2+	3+	4+
Carcinoma Cells	31 (34%)	14 (15%)	1 (1%)	11 (12%)	9 (10%)	25 (27%)
Normal Cells	93 (89%)	7 (7%)	4 (4%)	1 (1%)	0 (0%)	0 (0%)

EXAMPLE 4

[0069] This example illustrates endothelial receptor (gp60)-mediated caveolar transcytosis of paclitaxel albumin nanoparticles (ABI-007).

[0070] Paclitaxel (P) albumin nanoparticles (Abraxane, ABX or ABI-007) demonstrated improved response rate over Taxol in a phase III metastatic breast cancer trial (33% vs 19%, $p<0.0001$) (SABCS, O'Shaughnessy et al, 2003). Cremophor in Taxol (TAX) entraps P in micelles in plasma, reducing the paclitaxel available for cellular partitioning (see, e.g., Sparreboom et al., *Cancer. Res.*, *59*, 1454 (1999)). Studies in athymic mice have shown 30-40% higher intratumor paclitaxel concentrations with ABX as compared to equal doses of TAX (SABCS, Desai et al, 2003). Albumin is transported across endothelial cells (EC) by specific receptor (gp60)-mediated caveolar transport (see, e.g., John et al., *Am. J. Physiol.*, *284*, L187 (2001)). It was hypothesized that albumin-bound paclitaxel in ABX may be transported across tumor microvessel EC by gp60, and this mechanism may be particularly active for ABX as compared to TAX.

[0071] A series of experiments were performed to evaluate binding and transport of paclitaxel by human umbilical vein endothelial cells (HUVEC) and human lung microvessel endothelial cells (HLMVEC) for ABX and TAX. Fluorescent paclitaxel (FP) was used as a probe and fluorescent ABX and TAX were formulated with FP to probe the binding and transport of paclitaxel across EC monolayers grown on a transwell apparatus.

[0072] Binding of paclitaxel to cells (HUVEC) was 10X higher for ABX than TAX. The transport of paclitaxel from ABX across EC monolayers was enhanced by 2-3 fold and 2-4 fold for HUVEC and HMVEC, respectively, as compared to TAX. Transport was dependent on albumin. Transport of paclitaxel from ABX was inhibited by the presence of anti-SPARC antibody, which is known to bind gp60, the receptor required for caveolar albumin transcytosis. Known inhibitors of calveolar transcytosis, NEM and β-methyl cyclodextrin (BMC), also inhibited the transport of paclitaxel from ABX across the endothelial monolayers (Figure 2). Inhibition of caveolar transport decreased transport of P from ABX to the level of TAX transport.

[0073] These results demonstrate that paclitaxel from ABX is actively transported across EC by gp60-mediated caveolar transcytosis, whereas P from TAX appears to be transported at a 2-4 fold lower rate primarily by a paracellular (non-caveolar) mechanism. This pathway may in part be responsible for increased intratumoral concentrations of paclitaxel seen for ABX relative to TAX. Cremophor in TAX inhibits transcytosis of paclitaxel across endothelial cells.

EXAMPLE 5

[0074] This example demonstrates correlation of SPARC overexpression with high response rates using nanoparticle albumin-bound paclitaxel (ABI-007) in squamous head and neck cancers.

[0075] In phase I and II clinical studies of patients with squamous cell carcinoma (SCC) of head and neck (H&N) and anal canal, response rates of 78% and 64% were observed, respectively, for intra-arterially delivered Nanoparticle Albumin-Bound Paclitaxel (Abraxane, ABX or ABI-007) (see, e.g., Damascelli et al., *Cancer*, *92(10)*, 2592-2602 (2001), and Damascelli et al., *AJR*, *181*, 253-260 (2003)). In comparing *in vitro* cytoxicity of ABX and Taxol (TAX), we observed that a squamous cervix (A431) line demonstrated improved IC_{50}s for ABX (0.004 μg/ml) vs TAX (0.012 μg/ml). Albumin-mediated transendothelial caveolar transport of paclitaxel (P) and increased intratumoral accumulation of P for ABX vs TAX was demonstrated recently (see, e.g., Desai, SABCS 2003).

[0076] Human H&N tumor tissues (n=119) and normal human H&N tissue (n=15) were immunostained and scored (0-4) for SPARC staining using a tumor and normal tissue array. Immunostaining was performed using polyclonal rabbit anti-SPARC antibody. In a new phase I dose escalation study (ABX given IV over 30 minutes q3w), a subset of head and neck cancer patients (n=3) were analyzed for response to ABX.

[0077] SPARC was overexpressed (score ≥ 2) in 60% (72/119) of the H&N tumors versus 0% (0/15) in normal tissues ($p<0.0001$). In the phase I study, 2/3 H&N patients achieved partial response (PR) after 2 cycles of treatment at dose levels of 135 mg/m^2 (1 pt) and 225 mg/m^2 (1 pt). A third patient at 260 mg/m^2 progressed.

[0078] SPARC was found to be overexpressed in 60% of squamous H&N tumors. This may explain the high single-agent activity of ABX seen previously in squamous H&N cancers due to binding of albumin-bound paclitaxel to SPARC expressed in these tumors. 2/3 patients with squamous H&N tumors achieved PR in a new phase I study.

- SPARC Staining:

	0	-/+	1	2	3	4
H&N Tumor Array:						
– Carcinoma	17	14	16	23	20	29
	(14%)	(12%)	(13%)	(19%)	(17%)	(24%)
– Normal	13	0	2	0	0	0
	(87%)	(0%)	(13%)	(0%)	(0%)	(0%)

Human H&N tumor tissues (n=119) and normal human H&N tissue (n=15) were immunostained and scored (0-4) for SPARC staining using a tumor and normal tissue array. Immunostaining was performed using polyclonal rabbit anti-SPARC antibody. SPARC was overexpressed (score ≥ 2) in 60% (72/119) of the H&N tumors versus 0% (0/15) in normal tissues ($p<0.0001$). This may explain the high single-agent activity of ABX seen previously in squamous H&N cancers due to binding of albumin-bound paclitaxel to SPARC expressed in these tumors.

[0079] In a new phase I dose escalation study (ABX given IV over 30 minutes q3w), a subset of head and neck cancer patients (n=3) were analyzed for response to ABX. In the phase I study, 2/3 H&N patients achieved partial response (PR) after 2 cycles of treatment at dose levels of 135 mg/m^2 (1 pt) and 225 mg/m^2 (1 pt). A third patient at 260 mg/m^2 progressed. Tumor tissues from these patients were stained for SPARC and 1 responding patients showed strong overexpression for SPARC.

[0080] In another phase II clinical study of patients with squamous cell carcinoma (SCC) of head and neck (H&N) treated with intra-arterial Abraxane, an overall response rate of 68% was noted. In 10 responding patients whose tissues were analyzed for SPARC staining, 70% were found to strongly overexpress SPARC.

EXAMPLE 6

[0081] This example illustrates the internalization of labeled albumin into MX-1 tumor cells and colocalization within the MX-1 cell with intracellular SPARC expression.

[0082] MX-1 cells were cultured on a coverslip and permeabilized with suitable agents. Cells were exposed to fluorescent albumin and following washing were exposed to SPARC antibody. This was followed by exposure to a secondary antibodies having a different fluorescent tag than the albumin. It was surprisingly observed that the labeled albumin colocalised with the presence of SPARC within the cell indicating that albumin was rapidly internalized and targeted intracellular SPARC.

EXAMPLE 7

[0083] This example demonstrates an increase in endothelial transcytosis via gp60 (albumin receptor) of pharmaceutical compositions comprising paclitaxel and albumin as compared to Taxol.

[0084] Human lung microvessel endothelial cells (HLMVEC) were grown to confluence on a transwell. The inventive pharmaceutical composition comprising paclitaxel and albumin, or Taxol containing fluorescent paclitaxel (Flutax) at a concentration of 20 μg/mL, was added to the upper transwell chamber.

[0085] The transport of paclitaxel by transcytosis from the upper chamber to the lower chamber was monitored continuously using a fluorometer. A control containing only Flutax without albumin was also used. The control with Flutax showed no transport, validating the integrity of the confluent HLMVEC monolayer. Transport of paclitaxel from the albumin-paclitaxel composition was much faster than paclitaxel from Taxol in the presence of 5% HSA (physiological concentration). Transport rate constants (K_t) for the albumin-paclitaxel composition and Taxol were 1.396 h^{-1} and 0.03 h^{-1}, respectively. The total amount of paclitaxel transported across the monolayer was three times higher for the albumin-paclitaxel composition than Taxol. Thus, the use of albumin or other suitable mimetic including aantibodies or fragments against the gp60 recepetor or other endothelial cell receptor can assist in the transport of a desired therapeutic agent across the endothelial barrier into the tumor interstitium.

EXAMPLE 8

[0086] This example demonstrates the specific binding of anti-SPARC antibody to SPARC.

[0087] Whole cell extract was prepared from HUVEC cells by sonication. The protein was separated on a s5-15% SDS-PAGE, transferred onto PVDF membrane and visualized with a polyclonal antibody against SPARC and a monoclonal antibody against SPARC.

Both antibodies reacted to a single band at 38 kDa, the correct molecular weight for SPARC. When MX-1 was analyzed by the same method, SPARC was detected in both the clarified cell lysate or the membrane rich membrane fraction.

EXAMPLE 9

[0088] This example demonstrates the absence of SPARC expression in normal tissues.

[0089] Normal human and mouse tissue were immunostained and scored (0-4) for SPARC staining using a tumor and normal tissue array. Immunostaining was performed using polyclonal rabbit anti-SPARC antibody. SPARC was not expressed in any of the normal tissues, with the exception of the esophagus. Likewise, SPARC was not expressed in any of the normal mouse tissue, except the kidney of the female mouse. However, it is possible that this expression was due to follistatin which is homologous to SPARC.

SPARC Expression in Human Normal Tissues

Stomach	0/8
Colon	0/9
Rectum	0/15
Liver	0/14
Spleen	0/10
Lung	0/14
Kidney	1/14
Brain	1/14
Testis	0/8
Prostate	0/3
Heart	0/9
Tonsil	0/10
Lymph Nodes	0/10
Appendix	0/10
Esophagus	**5/5**
Pancreas	0/5
Eyeball	0/5
Ovary	0/5

Mouse Normal Tissues

Liver	0/19
Kidney (M)	0/8
Kidney (F)	**6/8**
Lung	0/16
Muscle	0/20
Brain	0/20
Heart	0/18

Stomach	0/20
Spleen	0/20

[0090] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0091] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0092] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED IS:

1. A method for determining the response of a mammalian tumor to a chemotherapeutic agent, the method comprising (a) isolating a biological sample from the mammal, (b) detecting the expression of SPARC protein in the biological sample, and (c) quantifying the amount of SPARC protein in the biological sample.

2. The method of claim 1, wherein the biological sample is isolated from the tumor.

3. The method of claim 1, wherein the biological sample is isolated from a bodily fluid.

4. The method of claim 3, wherein the bodily fluid is selected from the group consisting of cerebrospinal fluid, blood, plasma, serum, and urine.

5. The method of claim 1, wherein the tumor is located in the bladder, liver, ovary, kidney, gut, brain, or breast.

6. The method of claim 1, wherein the mammal is a human.

7. A method for delivering a therapeutic agent to a disease site in a mammal, the method comprising administering to the mammal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises the therapeutic agent coupled to a compound capable of binding an albumin-binding protein and a pharmaceutically acceptable carrier, provided that the pharmaceutical composition does not comprise more than 50% of the therapeutic agent in nanoparticle form.

8. The method of claim 7, wherein the pharmaceutical composition does not comprise more than 10% of the therapeutic agent in nanoparticle form.

9. The method of claim 7, wherein the pharmaceutical composition does not comprise more than 5% of the therapeutic agent in nanoparticle form.

10. The method of claim 7, wherein the albumin-binding protein is a SPARC protein.

11. The method of claim 7, wherein the disease site is a tumor.

12. The method of claim 7, wherein the therapeutic agent is a diagnostic agent.

13. The method of claim 12, wherein the diagnostic agent is selected from the group consisting of radioactive agents, MRI contrast agents, X-ray contrast agents, ultrasound contrast agents, and PET contrast agents.

14. The method of claim 7, wherein the compound is a ligand that binds the SPARC protein.

15. The method of claim 14, wherein the ligand is selected from the group consisting of a calcium cation (Ca2+), copper cation (Cu2+), iron cation (Fe2+), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), collagen I, collagen II, collagen III, collagen IV, collagen V, collagen IX, vitronectin, thrombospondin-1, endothelial cells, platelets, albumin, and hydroxyapatite.

16. The method of claim 7, wherein the compound capable of binding an albumin-binding protein is a small molecule.

17. The method of claim 16, wherein the small molecule is selected from the group consisting of a chemical, a protein, and a carbohydrate.

18. The method of claim 7, wherein the compound capable of binding an albumin-binding protein is an antibody to the SPARC protein.

19. The method of claim 11, wherein the tumor is located in the bladder, liver, ovary, kidney, gut, brain, or breast.

20. The method of claim 7, wherein the mammal is a human.

21. The method of claim 7, wherein the route of administration is selected from the group consisting of intravenous, intraperitoneal, intratumoral, and inhalational.

22. The method of claim 18, wherein the antibody is a polyclonal antibody.

23. The method of claim 22 wherein the antibody is manufactured in an animal selected from the group consisting of a chicken, rat, mouse, rabbit, guinea pig, hamster, sheep, cow, and goat.

24. The method of claim 18, wherein the antibody is humanized.

25. The method of claim 18, wherein the antibody is monoclonal.

26. The method of claim 25, wherein the antibody is manufactured by fusion of a single cell line producing anti-SPARC antibody with a myeloma line.

27. The method of claim 18, wherein the antibody is made in vitro by a method selected from the group consisting of a peptide synthesizer, by conversion of display phage into antibody, and mutagenesis or synthesis of variable regions of the heavy and light chain genes for antibody.

28. The method of claim 18, wherein the antibody is selected from the group consisting of monovalent, multivalent; monospecific, bispecific, single chain, and a Fab fragment.

29. The method of claim 27, wherein the antibody is a chimera.

30. The method of claim 18, wherein the therapeutic agent is selected from the group consisting of tyrosine kinase inhibitors, kinase inhibitors, biologically active agents, biological molecules, radionuclides, adriamycin, ansamycin antibiotics, asparaginase, bleomycin, busulphan, cisplatin, carboplatin, carmustine, capecitabine, chlorambucil, cytarabine, cyclophosphamide, camptothecin, dacarbazine, dactinomycin, daunorubicin, dexrazoxane, docetaxel, doxorubicin, etoposide, epothilones, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine, mechlorethamine, mercaptopurine, meplhalan, methotrexate, rapamycin (sirolimus), mitomycin, mitotane, mitoxantrone, nitrosurea, paclitaxel, pamidronate, pentostatin, plicamycin, procarbazine, rituximab, streptozocin, teniposide, thioguanine, thiotepa, taxanes, vinblastine, vincristine, vinorelbine, taxol, combretastatins, discodermolides, transplatinum, anti-vascular endothelial growth factor compounds ("anti-VEGFs"), anti-epidermal growth factor receptor compounds ("anti-EGFRs"), 5-fluorouracil and derivatives and combinations thereof.

31. The method of claim 30, wherein the therapeutic agent is a kinase inhibitor.

32. The method of claim 31, wherein the kinase inhibitor is genistein.

33. The method of claim 30, wherein the therapeutic agent is a biological molecule.

34. The method of claim 33, wherein the biological molecule is selected from the group consisting of tTF and TNF.

35. The method of claim 18, wherein the therapeutic agent is a radionuclide.

36. The method of claim 18, wherein the therapeutic agent is an antibody which mediates complement activation and cell mediated cytotoxicity against the tumor.

37. The method of claim 18, wherein the tumor is located in the bladder, liver, ovary, kidney, gut, brain, or breast.

38. The method of claim 18, wherein the mammal is a human.

39. The method of claim 18, wherein the route of administration is intravenous, intraperitoneal, intratumoral, or inhalational.

40. A composition comprising a therapeutic agent coupled to a compound capable of binding a SPARC protein and a pharmaceutically acceptable carrier, provided that the composition does not comprise more than 50% of the therapeutic agent in nanoparticle form.

41. The composition of claim 40, wherein the compound capable of binding the SPARC protein is an antibody or fragment thereof.

42. The composition of claim 41, wherein the antibody or fragment thereof is humanized and is selected from the group consisting of monovalent, multivalent, monospecfic, bispecific, single chain, monovalent Fab', divalent Fab2, scfv, diabody, and a chimera.

43. The composition of claim 41, wherein the therapeutic agent is a chemotherapeutic, radionuclide, or peptide.

44. The composition of claim 41, wherein the antibody is polyclonal.

45. The composition of claim 44, wherein the antibody is manufactured in an animal selected from the group consisting of a chicken, rat, mouse, rabbit, guinea pig, hamster, sheep, cow, and goat.

46. The composition of claim 45, wherein the antibody is humanized.

47. The composition of claim 41, wherein the antibody is monoclonal.

48. The composition of claim 47, wherein the antibody is manufactured by fusion of a single line producing anti-SPARC antibody with a myeloma line.

49. The composition of claim 41, wherein the antibody is made in vitro by a method selected from the group consisting of a peptide synthesizer, by conversion of display phage into antibody, and mutagenesis or synthesis of variable regions of the heavy and light chain genes for antibody.

50. The composition of claim 41, wherein the therapeutic agent is selected from the group consisting of tyrosine kinase inhibitors, biologically active agents, radionuclides, adriamycin, ansamycin antibiotics, asparaginase, bleomycin, busulphan, cisplatin, carboplatin, carmustine, capecitabine, chlorambucil, cytarabine, cyclophosphamide, camptothecin, dacarbazine, dactinomycin, daunorubicin, dexrazoxane, docetaxel, doxorubicin, etoposide, epothilones, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine, mechlorethamine, mercaptopurine, meplhalan, methotrexate, rapamycin (sirolimus), mitomycin, mitotane, mitoxantrone, nitrosurea, paclitaxel, pamidronate, pentostatin, plicamycin, procarbazine, rituximab, streptozocin, teniposide, thioguanine, thiotepa, taxanes, vinblastine, vincristine, vinorelbine, taxol, combretastatins, discodermolides, transplatinum, anti-vascular endothelial growth factor compounds ("anti-VEGFs"), anti-epidermal growth factor receptor compounds ("anti-EGFRs"), 5-fluorouracil and derivatives and combinations thereof.

51. The composition of claim 41, wherein the therapeutic agent is a kinase inhibitor.

52. The composition of claim 41, wherein the therapeutic agent is a biological molecule selected from the group consisting of tTF and TNF.

53. The composition of claim 41 wherein the therapeutic agent is a radionuclide.

54. The composition of claim 41, wherein the therapeutic agent is an antibody which mediates complement activation and cell mediated cytotoxicity against the tumor.

55. The composition of claim 41, wherein the route of administration is selected from the group consisting of intravenous, intraperitoneal, intratumoral, or inhalational.

56. A delivery agent comprising a SPARC recognition group and a therapeutic agent, wherein the therapeutic agent is coupled to the SPARC recognition group, provided

that the delivery agent does not comprise more than 50% of the therapeutic agent in nanoparticle form.

57. The delivery agent of claim 56, wherein the SPARC recognition group comprises an antibody or fragments thereof selected from the group consisting of monovalent Fab', divalent Fab2, scfv, diabody, a chimera antibody or fragments thereof, and wherein the therapeutic agent is a chemotherapeutic, radionuclide, peptide, cytotoxic agent, kinase inhibitor, biological molecule, or diagnostic agent and combinations thereof.

58. The delivery agent of claim 56, wherein the SPARC recognition group is a humanized antibody or fragment thereof.

59. A method for delivering a chemotherapeutic agent to a tumor in a mammal, wherein the method comprises administering to a mammal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises a chemotherapeutic agent coupled to a SPARC protein capable of binding albumin and a pharmaceutically acceptable carrier.

60. The method of claim 59, wherein the SPARC is expressed from a virus selected from bacculovirus or adenovirus.

61. The method of claim 59, wherein the SPARC is purified using chromatography.

62. The method of claim 61, wherein the chromatography is selected from the group consisting of ionic, size exclusion, or C-18 chromatography.

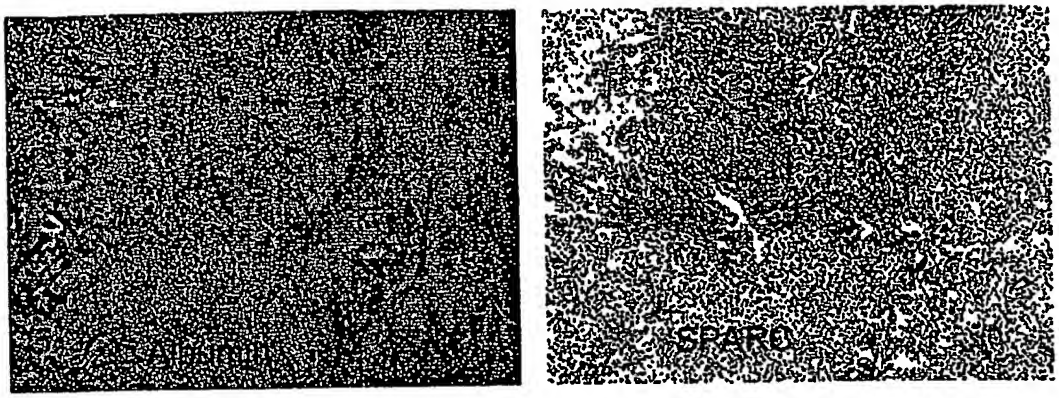
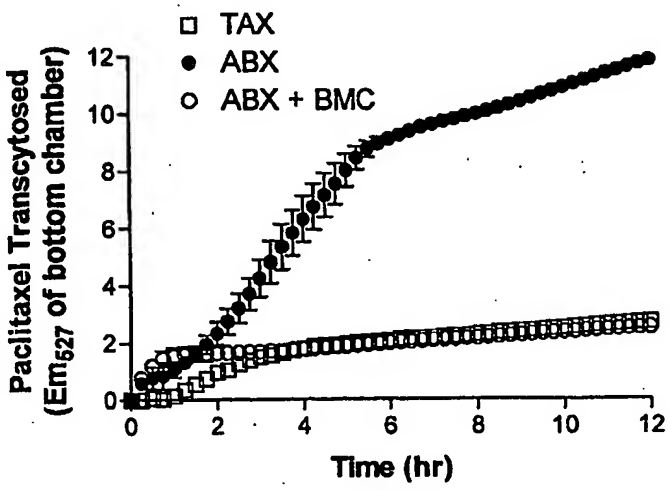


Figure 1. Colocalization of albumin and SPARC in serial sections of an MX-1 xenograft tumor.

Figure 2. Transcytosis of Paclitaxel across Endothelial Cell Monolayers



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(54) Title: TREATMENT METHODS UTILIZING ALBUMIN-BINDING PROTEINS AS TARGETS

(57) Abstract: The invention provides a method for determining the response of a mammalian tumor to a chemotherapeutic agent comprising detecting and quantifying the SPARC protein in a sample isolated from the human. The invention also provides a method for delivering a chemotherapeutic agent to a tumor in a human that expresses SPARC comprising administering a pharmaceutical composition comprising the chemotherapeutic agent coupled to a compound that binds SPARC protein. The invention further provides a composition comprising a chemotherapeutic agent coupled to a compound capable of binding SPARC protein and a pharmaceutically acceptable carrier.

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,T,E	US 2005/0176669 A1. (AL-MURRANI) 11 August 2005 (11.08.05), see entire. document.	1-62

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